PROPAGATION AND UTILIZATION OF GRAFTED TOMATOES IN THE GREAT PLAINS

by

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Abstract

Grafting with inter-specific hybrid rootstock is effective for tomato (*Solanum lycopersicum*) growers looking to reduce soilborne disease organically and increase fruit yield in the Southeastern US. However, production with grafted tomatoes has not been tested in the Great Plains region of the US. Small-acreage growers would like to produce grafted plants themselves, but many have difficulty with propagation due to water stress in the scion post-grafting and/or high temperatures within healing chambers. Growers may be able to reduce water stress post-grafting by removing the upper portion of the shoot to reduce leaf surface area, but no data exist on the potential effects of this practice on mature plant yield. Five high tunnel and one open-field study were conducted in 2011 and 2012 to investigate yield effects related to the use of two rootstocks and shoot removal during the grafting procedure. Grafting significantly increased fruit yield in five of the six trials ($P<0.05$). The average yield increases by Maxifort and 'Trooper Lite' rootstocks were 53% and 51%, respectively, across all trials. In some trials shoot removal during the grafting process reduced yield and could depend upon rootstock vigor. Another series of experiments were performed testing the efficacy of shoot removal for graft survival during the healing period prior to field planting. Five healing chambers designs were evaluated, and no significant effects of treatment design were observed upon grafted seedling survival. Plants grafted with no chamber had success rates of 81% to 91%. Additionally, three grafting leaf removal techniques were studied, and a partial leaf removal method had significantly higher success rates as compared to fully foliated and defoliated plants ($P<0.05$). Partial leaf removal may be recommended as a way to reduce water stress in the plant, and could potentially be a way to simplify the grafting process for small-scale producers.

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Dedication

"I believe a leaf of grass is no less than the journey-work of the stars…"

-W.W.
Verse 31, *Song of Myself*

I dedicate this work to my parents, Michael and Jane Ellen, and to my extraordinary friend Diane Schneider, who encouraged and supported me every step of the way.

Chapter 1 - Review of the Literature

Herbaceous Grafting as a technology and IPM strategy for Tomatoes

History and Significance of Grafting

The domestication and cultivation of food sources gave rise to human settlement and ultimately modern civilization. Humans have sought methods to improve crop success for several millennia. Grafting, an ancient technique of unknown specific historical origin, was developed to improve production in woody plants by means of growing the vascular systems of two related species. Ultimately, grafting improves yield quality and volume via the union of desired qualities from two initially separate plant bodies. Despite the application of this technique originating with fruit trees, grafting may be employed with vegetable – specifically solanaceous and cucurbitaceous – crops in order to improve yield and combat soilborne pathogens. Furthermore, the resurgence in growers' interest in organic cultivation practices and mandated phaseout of some soil fumigants have made vegetable grafting a major topic of interest in the horticultural community throughout the past decade (Davis et al, 2008; Kubota et al., 2008, Louws et al., 2010).

The origin of grafting is often attributed to Chinese horticulturalists; though the specific date is still disputed, records suggest grafting was being utilized in China for fruit trees by 1500 BC (Hartman et al., 2002). Both Aristotle (384-322 BC) and his successor Theophrastus (371-287 BC) wrote about the issue of graft compatibility, a continued challenge for modern horticulturalists. The ancient Roman poet Virgil included a lovely description of grafting in his pastoral "Georgics":

But various are the ways to change the state

Of plants, to bud, to graff, to inoculate
For, where the tender rinds of trees disclose
Their shooting germs, a swelling knot there grows:
Just in that space a narrow slit we make,
Then other buds from bearing tress we take;
Inserted thus, the wounded rind we close,
In whose moist womb the admitted infant grows.
But when the smoother bole from knots is free;
We make a deep incision in the tree.
And in the solid wood the slip inclose;
The battening bastard shoots again and grows;
And in short space the laden boughs arise;
With happy fruit advancing to the skies.
The mother plant admires the leaves unknown
Of alien trees and apples not her own (Virgil, 1953).

Woody grafting became a common practice throughout the Roman Empire, and the practice reportedly survived the Dark Ages both on the fringes of the European continent in Islamic gardens and from within at Christian monasteries (Mudge et al., 2009). The revival of the Renaissance solidified the utilization of woody grafting as the customary international practice it is today. According to the seminal grafting tome of the era, ‘‘there is none that more doth refresh the vital spirits of men, nor more engender admiration in the effects of nature, or that is cause of greater recreation to the weary and traveyled spirit of man, or more profitable to mans life, than is the skill of planting and graffing [*sic*]’’ (Mascall 1589).

Vegetable grafting, however, has a much briefer and somewhat less poetic history. Related in theory to woody grafting, the basic process of vegetable grafting employs a hybridized rootstock resistant to soilborne diseases and a scion of a less-resistant variety that produces desirable fruit, etc. (Grubinger 2007). Herbaceous grafting was developed in Asia during the early 1920s to combat soilborne disease in melons (Ashita 1927; Lee 1994), and it has since garnered popularity in regions with intensive land use and small agricultural areas (Hartmann and Kester, 1975). Overall, herbaceous grafting has an increasing presence in worldwide cultivation practices. European grafted transplant production has steadily grown with Spain leading the helm (129.8 million annually), and Italy and France (47.1 million and 28 million, respectively) following (Lee et al., 2010; Morra and Bilotto, 2009).

The majority of Korean and Japanese vegetable cultivation utilizes grafting, with approximately 700 million seedlings being produced in each country in 2008 (Lee et al., 2010). However, a mere estimated 40 million grafted tomato seedlings are used annually in North American greenhouses (Kubota et al., 2008; Rivero, R. M., J. M. Ruiz, et al. 2003a). Vegetable grafting has a burgeoning market with plenty of demand in the United States, and research and experimentation will certainly streamline and popularize the use of this technique for American growers and high tunnel producers (Kubota, et al., 2008). The added benefits vegetable grafting offers, specifically with high-value crops such as tomato (*Solanum lycopersicum*) indicate that this cultivation practice may be a profitable enterprise.

Grafting Benefits: Stress Control and Crop Productivity

It is well documented that growers graft high-value crops to increase overall vigor, yield, tolerance to abiotic stress and disease resistance (Lee, 1994, 2003, 2007; Paroussi et al., 2007; Rivard and Louws, 2008, Lee et al., 2010). The original and primary function of grafting is to combat soilborne agricultural diseases with significant yield gain in grafted plants (Louws et al., 2010; Marsic and Osvald, 2004).

The chief fungal, bacterial, and nematode diseases that many tomato varieties are susceptible to include: fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*), verticilium wilt (*V.*

dahliae), root-knot nematodes (*Meloidogyne spp*.) and southern stem blight (*Sclerotium rolfsii)* (Rivard and Louws, 2008; Miller et. al. 2009; Louws et al., 2010). *Fusarium oxysporum* commonly colonizes the plant xylem via infection of the roots and affects over one hundred herbaceous species (Miller et. al. 2009). Perhaps far more severe, verticillium wilt comes in at least two aggressive forms, can live in soil for up to fifteen years, and affects over three hundred herbaceous species. Also difficult to combat once established in soils, root-knot nematodes include a large number of globally distributed species consisting of microscopic worms that penetrate roots and create debilitating root "galls" that prevent nutrient and water absorption (a further monetary loss for the farmer and inhibit the plant from coping with environmental stresses); additionally, it has been estimated that the sixty international species of genus *Meloidogyne* has contributed to about 5% of global crop loss (Sasser and Carter, 1985). Also globally distributed, *Ralstonia* soil bacteria affects many crops (i.e. bananas, tomatoes, potatoes, tobacco) and is highly mobile, as it is spread by contaminated tools, irrigation water, soil, and infected seed. Southern stem blight fungus is common in humid regions and promptly kills the affected plant, typically tomatoes, peppers, and southern peas (Killebrew, 1997).

The most common traditional treatment for these soilborne diseases is the application of broad-spectrum pesticides, primarily methyl bromide until recently. Although it successfully disinfests the soil of most of the aforementioned diseases, the implications of a broad-spectrum pesticide such as methyl bromide are vast and cause for international re-evaluation of disease management practices.

Although grafting is useful for managing soilborne diseases, many of them are not common at high frequencies for Midwestern growers, as they are not as established in the region compared to other areas of the United States due to cropping history. Therefore, a major point of interest in the case for herbaceous grafting is that of increasing crop productivity. Specifically, yields are increased in grafted watermelon and cucumber (Pavlou, Vakalounaki, and Ligoxigakis, 2002; Upstone, 1968). Tomato grafting usually leads to increased fruit yield via larger fruit size (Pogonyi at al., 2005; Augustin, Graf, and Laun, 2002). This increase in output vigor is linked to heightened rootstock growth, thereby affecting water uptake and nutrient content (Leonardi and Giuffrida, 2006; Ruiz, Belakbir, and Romero, 1996; Fernandez-Garcia et al., 2002).

Grafting as an Alternative to Soil Fumigation

For decades, soil fumigation was a method of biotic stress control for high value crops, and a popular fumigant was methyl bromide. Developed in 1932, methyl bromide (also known as bromomethane) is a broad-spectrum fumigant employed as an herbicide, insecticide, nematicide, acaricide, fungicide, and rodentcide (NPIC 2000); moreover, the colorless and odorless gas volatilizes upon application and disperses to the atmosphere, where it wreaks havoc upon ozone bonds. The toxicity – as a biocide and an ozone depletor – of this product led to its phaseout under the guidelines of the Montreal Protocol. Since then many mid-size to larger scale growers sought alternatives to soil fumigation.

In order to aid growers in this transition, The United Nations Environment Program Division of Technology, Industry, and Economics (UNEP TIE) funded research projects targeting alternative IPM techniques. Though the United States has not completely reached a complete phase-out of methyl bromide, usage levels have been dramatically reduced since implementation of the program. However, developing nations lack much of the funding and administrative organization to rapidly reduce dependence upon such an effective, indirectly destructive pesticide.

A seminal research project was conducted in Morocco under direction of Dr. Mohamed Besri, and grafting was evaluated as a potential phase-out technique. The Moroccan agricultural economy relies heavily on exporting tomatoes, which represents 63% of national vegetable production and 22% of agricultural employment (Porter, 2001). Because of this, methyl bromide soil fumigation increased 40-fold from 1989 to 1998 in Morocco, and tomato fields/greenhouses represent 58% of the total area of Moroccan land fumigated with methyl bromide. Pesticide application was targeted at root-knot nematodes, which are particularly prevalent in this region, as well as fungal wilts. This grafting IPM program was introduced in 1992 on a number of small-scale farms. In the UNEP study, appropriate rootstock cultivars were chosen to resist the aforementioned soilborne diseases. Traditional farming with methyl bromide application cost \$61,585 in a year, the grafted plant study cost \$64,825 – a 5% increase (Porter, 2001).

In 2003 Besri followed up these numbers with a study of exportation rates and disease resistance in the same areas. Essentially, grafting proved to enhance soilborne pathogen control, increased salt tolerance, increase in fruit yield (Rivard and Louws, 2008), as well as fruit quality (Besri 2003). Follow-up research showed in a comparative study of 20,000 non-grafted plants to 10,000 grafted ones, nematode galling index reduced by 75% in grafting with resistant rootstocks; additionally, Besri measured a 10 to 14% greater fruit yield in grafted plants with 5% greater grafted fruit exportation (an expression of fruit quality) (Besri 2003). Finally, although the extra cost of grafted plants was roughly \$2,500 greater than traditional management, Besri measured the total net profit gain of grafted plants to be \$5,610 more than that of the non-grafted plants. In this study, large-scale grafting processes were the best solution to methyl bromide application and could enhance profits in the agricultural sector.

Thus, this successful series of studies in a developing nation such as Morocco with a minimal increase in grafting costs and a significant return suggests that grafting is a highly viable IPM strategy internationally. While few horticultural growers fumigate for biotic stress control in the Midwestern US, the boon of grafting in this case study in Morocco may aid mid-size to large-scale tunnel and field growers in selecting IPM strategies.

Grafting in High Tunnels

High tunnels are slow to popularize in the United States when compared to their use in other countries (Emmert, 1955; Wittwer, 1993). These passively heated structures require extensive irrigation and a significant start-up cost (Lamont et al., 2003), which may deter small-scale growers from adoption of this technology. Researchers and extension specialists strive to find high tunnel and open field planting combinations for tomato that maximize yield for producers (Taber et al., 2007; Carey et al., 2009). Wells and Loy were among the first researchers in the United States to tout the benefits of high tunnel crop cultivation, with a particular focus on tomato (1993). Not only do high tunnels extend the growing season anywhere from four to ten weeks (Wells and Loy, 1993; Hunter, Drost, and Black, 2010), they also increase yield, economic return, and marketing opportunities. One study occurring in western Washington found that although high tunnel tomato production was eight times more costly than open field production, the high-tunnel grown tomatoes were three times as profitable (Galinato and Miles, 2013). A study in North Carolina suggested that the reimbursement period for high tunnel start up ranged anywhere from two to five years (Sydorovych et al., 2013). Another study in North Carolina achieved 33% larger yields in high tunnel tomato cropping systems compared to open field systems (O'Connell et al., 2012). A recent survey found that high producer interest in high tunnel adoption exists in the United States, as growers rush to meet the growing demand for fresh and locally-grown produce; however, this same survey estimates that a mere three acres of Kansas farmland is under high tunnel cultivation for tomatoes, leafy greens, peppers, and cut flowers (Carey et al., 2009). Since this time, a cost-share program led by the National Resource Conservation Service has been aiding growers in the adoption of high tunnels and more than 350 tunnels have been built in the state of Kansas alone since 2009 (Banks, personal communication). High producer interest, general construction affordability, and the profitability of high value crops make high tunnels a significant tool for tomato production (Carey et al., 2009), and implementing grafting technology within these systems may further increase yield and market return.

Internationally, grafting is often employed in intensive cropping systems such as nursery greenhouses and high tunnels (Lee et al., 1994). This is due to the performance of grafted plants, as tolerant rootstock has been shown to overcome multiple abiotic stressors such as salinity (Cuartero et al., 2006; Estan et al., 2005; Rivero et al., 2003a), thermal stress (Abdelmageed et al., 2004), and excessive soil moisture (Black et al., 2003) that are associated with heated, successively cropped areas like high tunnels and greenhouses (Lee et al., 2010; Hoyos, 2010; Lee, 1994; Lee and Oda, 2003). Yet grafted vegetables still are not as widely used in North America as they are in other parts of the world, and growers – both large and small-scale – seek recommendations (Kubota et al. 2008). However, the potential for propagators to capitalize on this relatively untapped market exists. An economic analysis of grafted seedling production demonstrated that propagators may successfully market plants that cost nearly 150% more than non-grafted plants, ultimately compensating for any added labor and material costs for the grafting process (Rivard et al., 2010c). This same study demonstrated that small-scale growers may also graft their own seedlings with profitable results (Rivard et al., 2010c). Significant yield increases in grafted tomato are well documented (Upstone, 1968; Rahman et al., 2002; Ibrahim et al., 2001). The expansion of retail markets for grafted plants as well as the added benefits of high tunnel cultivation may make the combination of these two technologies especially profitable for high value crops.

Grafting Process for *Solanum lycopersicum*

Tube grafting

Several types of grafting styles may be employed in tomato production (Lee, 2003), but tube grafting – otherwise known as top grafting or splice grafting – may be considered the most popular grafting technique for tomato (*Solanum lycopersicum*). Researchers and producers agree that tube grafting is simple and efficient, as producers may be able to graft two to three times more plants at a time compared to the utilization of other grafting techniques (Oda, 2007; Hartmann et al., 2002; Lee, 2007; Rivard and Louws, 2006). Since scion and rootstock vascular bundles fuse together, the strengthened graft may also withstand more vigorous post-graft/pre-planting handling (Lee et al., 2010). Furthermore, international commercial growers tout tube grafting as an ideal grafting technique since the typical success rates range from eighty-five to ninety-five percent (Oda, 1995).

Some variation concerning the tube grafting procedure exists, but in general, the process begins when the plants are small (2-4 mm stem diameter). The rootstock, usually a compatible inter-specific hybrid that has been bred for increased disease resistance or nutrient uptake, and the scion, the plant body producing the desired fruit load, must be at similar stem diameters when the grafting procedure occurs. The rootstock and scion are then cut at approximately 60-75 degree angles, lined up, and then held together with a silicon clip. It is recommended to wear gloves and use sterilized scalpels (or hobby knives) when grafting to prevent the entrance of pathogenic bacteria like *Clavibacter michiganensis* to the graft union. The environment must then be altered to prevent water loss and wilt in the scion (Rivard and Louws, 2008; Davis et al., 2008). The specifications of these altered environments – otherwise known as healing chambers – for grafted seedlings will be discussed in the next section.

Physiologically, the time between the initial grafting day (Day 0) and the fourth day following the procedure is critical for seedling survival. During this early healing period, the scion is susceptible to wilt and death, as vascular tissue has yet to connect to the rootstock. Between Day 0 and 4, a wound reaction occurs via the death of damaged cell layers (Moore, 1984; Tiedemann, 1989), and then by the generation of a parenchymatous callus that fills in the gap between the rootstock and scion (Fernandez-Garcia, Carvajal, and Olmos 2004). This callus is believed to form from other undamaged cells at the graft site; these cells become hypertrophic and divide (Fernandez-Garcia, Carvajal, and Olmos 2004; Jeffree and Yeoman, 1983). Following this response, new vascular tissue develops from the surrounding meristematic tissue; the formation of this tissue is crucial, as water flow is integral to plant growth, mineral nutrition, and photosynthesis. Studies show that the graft union is not a barrier to hydraulic conductance through the plant (Fernandez-Garcia et al., 2002) and the graft union is considered to be functional with full use of hydraulic connectivity six to eight days after the grafting procedure (Turquois and Malone, 1996). Xylem vessels lignify via peroxidase and catalase enzymes, and one study suggests that the entire healing process is complete by Day 15 (Fernandez-Garcia, Carvajal, and Olmos 2004). This timeline is subject to slight variation due to the influence of environmental conditions at the time of grafting.

Healing chamber design and transpiration reduction techniques

Once the plants are grafted, they are moved into a healing chamber and there are numerous challenges to managing the healing chamber. The healing period is perhaps the most critical stage in the grafting process, as the plants are highly susceptible to any number of diseases, wilt, and the failure to heal. The process includes regulating the relative humidity (RH) as well as the amount of sunlight permeating the chamber at any given time in the healing process in order to maintain a low vapor pressure deficit (vpd) around the seedlings. The concept of potential evapotranspiration implies that plants passively transport water from the soil to the atmosphere, but plants may employ methods of internal resistance such as leaf rolling and stomatal closure to avoid significant water loss (Kirkham, 2005; Hsiao, 1973). Low temperature and high humidity are necessary for a low vpd and thereby a lessened rate of scion evapotranspiration.

Generally, plants are subjected to a period of acclimatization while the scion and rootstock heal together. The traditional recommendation for healing chamber design is a shaded plastic dome kept at a constant temperature (30°C) and high RH (>95%) via a cool-mist humidifier for the initial three to five days (McAvoy, 2005; Rivard and Louws, 2006). After approximately five days within the chamber, plants are gradually reintroduced to sunlight via partial shading and are weaned from the humidifier. Finally, it is important that the adventitious roots growing from the scion are trimmed to ensure the integrity of benefits received from the rootstock (McAvoy, 2005), and the plants should be left open to the greenhouse environment to 'harden off' prior to transplanting. All of these actions are preformed in order to permit the scion to photosynthesize under little stress while the seedling stems fuse.

There is some contention in the scientific community concerning the standardization of healing chamber management in the United States, as it can be rather problematic (Groff 2009; O'Connell et al., 2009). For example, a recent study at Washington State found that healing chamber dimensions affect relative humidity and internal air volume of the chambers, subsequently affecting graft survival rates (Johnson and Miles, 2011). The same study found a humidifier may not be necessary to ensure significant graft survival. Furthermore, Johnson and Miles suggest that grafted tomato are more tolerant of variable temperatures and lower RH than other crops (2011).

Due to the relative hardiness of tomato throughout the grafting process, reduction of leaf area may be possible for successful grafts. Preliminary work suggests that by reducing leaf area by eliminating the shoots of the scions, reliance on the healing chamber can be greatly reduced or eliminated altogether. Not only is healing chamber management difficult, but it also adds to the cost of producing a grafted transplant due to additional materials and labor (Rivard et al., 2010c).

Boosted photosynthetic rates have been observed in plants with partial defoliation (Hodginkinson, 1974), and a logarithmic increase in evapotranspiration is associated with greater leaf area indices – the proportion of leaf area above a unit area of the ground (Kirkham, 2005; Monteith, 1973). Total or partial scion defoliation may bypass the compulsion of a healing chamber during the grafting timeline, thereby reducing overall grafting maintenance and increasing grafting capabilities for small-scale growers. However, leaf removal/scion decapitation may have negative implications upon mature plant biomass and yield production (Decoteau, 1990).

This project seeks to conduct a research and extension program that will provide support and answer relevant questions related to post-grafting management as the United States propagation industry continues to progress in this area. Grafted propagation techniques have not been explored in a comprehensive way and there is a strong need for determining healing chamber management and post-grafting environment manipulation systematically.

Summary of Research Objectives

This work elaborates upon two experiements designed to analyze different facets and effects of tomato grafting. The first experiment (Chapter 2) aimed to determine the efficacy of two rootstock cultivars at increasing tomato fruit yield in high tunnels as well as test the effect of scion shoot removal upon mature plant yield and biomass. The second experiment (Chapter 3) focused upon the pre-planting grafting procedure by determining how healing chamber design (supplemental humidity and covering) affects graft survival; determining how healing chamber design affects the healing chamber environment; and determining how scion shoot removal affects graft survival in different healing chambers. Overall, the goal of this research is to investigate and streamline the tomato grafting in order to enhance the effectiveness and utilization of this technology.

Chapter 2 - The effect of scion shoot removal and rootstock cultivar on the yield of grafted tomatoes in high tunnels in the Great Plains

INTRODUCTION

Many small-scale and/or organic vegetable growers are implementing high tunnels as a way to reduce foliar disease and extend the growing season (Carey et al., 2009). In particular, tomatoes are a popular crop for high tunnels. However, managing soilborne diseases such as root-knot nematode (*Meloidogyne sp.*), southern blight (*Sclerotium rolfsii*), and Fusarium wilt can be very difficult and costly in these systems because of limited opportunity for crop rotations and therefore increased disease presence in the soil. In the southeastern US, where these diseases are very common, using disease-resistant rootstocks has been shown to be an effective integrated pest management (IPM) strategy to reduce root-knot nematodes (Rivard et al., 2010b), southern blight (Rivard et al., 2010b), fusarium wilt (Rivard and Louws, 2008), and verticillum wilt (Groff, 2009; Louws et al., 2010). However, there is little information focused upon grafting in production settings where less disease pressure exists, particularly in the Great Plains growing region of the central United States. Tomato production in high tunnels in the Midwest is increasing (Carey et al., 2009), and researchers have attempted to find high tunnel and open field planting combinations for tomato that maximize yield for producers (Taber et al., 2007; Carey et al., 2009). Not only do high tunnels extend the growing season anywhere from four to ten weeks (Wells and Loy, 1993; Hunter, Drost, and Black, 2010), they also increase yield, economic return, and marketing opportunities (Wells, 1991). A study in North Carolina achieved 33% larger yields in high tunnel tomato cropping systems compared to open field systems (O'Connell et al., 2012). High producer interest, general construction affordability, and the profitability of high value crops make high tunnels a significant tool for tomato production (Carey et al., 2009), and using grafted plants may further increase yield and market return.

Despite potential advantages and increasing grower interest, there is little market availability of grafted tomato plants propagated in the United States, and more than 30 million grafted tomato plants are currently imported into the United States from specialty nurseries in Canada (Kubota et al., 2008). Recently, herbaceous grafting nurseries have begun to appear in the United States, but very few if any are scaled for sale to commercial growers or have difficulties with the specialty requirements (such as specific rootstocks, specific scion, etc.) of small to mid-size growers. Rootstock selection may be essential to growers' grafting needs, and certain rootstocks may target specific diseases, abiotic pressures, or overall yield benefits. Two commercially available rootstock cultivars Maxifort (De Ruiter Seeds, Bergschenhoek, The Netherlands) and 'Trooper Lite' (Seedway, Hall, NY) may be utilized, as both are hybridized to resist disease pressure. Maxifort is a popular and very vigorous rootstock, while 'Trooper Lite' is a less vigorous, less common option. These two rootstocks were selected from the plethora of cultivars on the market for comparison in the subsequent field experiments.

Although growers can perform their own grafting, managing the grafted plants can be difficult (Groff, 2009; O'Connell et al., 2009). Grafted plants are usually placed inside "healing chambers" to maintain high humidity and reduce light intensity (Rivard et al., 2010c). Plastic healing chambers built inside greenhouses can overheat, leading to plant wilting and death. Healing chambers also add to the cost of producing a grafted transplant, as they require additional materials and labor (Rivard et al., 2010c). Reducing leaf area could reduce water stress and reduce the need for humidity management. This method is commonly used with ornamental and woody plants (Christopher, 1954; Harris, 2003). Reducing leaf area via elimination of scion shoots, reliance on the healing chamber can be greatly reduced or eliminated altogether. However, there is little information available as to whether this process scion shoot removal will affect tomato yield and fruit quality in a production setting. Therefore, there were two primary objectives for this research: (i) to determine the efficacy of two rootstock cultivars at increasing tomato fruit yield in high tunnels; and (ii) to test the effect of scion shoot removal upon mature plant yield and biomass.

MATERIALS AND METHODS

Transplant production and grafting

All grafted and nongrafted transplants were produced at the Throckmorton Plant Sciences Center at Kansas State University (Manhattan, KS; http://www.hfrr.ksu.edu/p.aspx?tabid=38). Scion and nongrafted cultivars were grown from commercially available 'BHN 589' seed (Siegers Seed Company, Holland, MI) and 'Cherokee Purple' (Johnny's Selected Seeds; Winslow, ME). 'BHN 589' is a determinate variety popular with high tunnel growers with fusariam wilt, verticillium wilt (race 1), and root-knot nematodes. 'Cherokee Purple' is a commonly grown indeterminate heirloom variety with no known resistance to soilborne pathogens. Commercially available rootstock cultivars Maxifort (De Ruiter Seeds, Bergschenhoek, The Netherlands) and 'Trooper Lite' (Seedway, Hall, NY) were selected as rootstock for the grafted treatments. Maxifort carries resistance against fusarium wilt (races 1 and 2), root-knot nematodes, tobacco mosaic virus, and verticillium wilt (race 1). 'Trooper Lite' confers resistance to: fusarium crown/root rot, fusarium wilt (race 2), tomato mosaic Virus, root-knot nematodes and corky root (*Rhizomonas suberifaciens)*.

In all trials a nongrafted control treatment was included as a standard comparison. All treatments were grafted via the Japanese tube grafting technique (Rivard et al., 2010c). Rootstock and scion seedling stems were cut and held together with a silicon clip. In the case of the shoot removal treatments (SR), shoot biomass (5-10 mm above scion cotyledons) was removed during the grafting procedure (**Figure 2.1**). All grafted seedlings were subsequently placed inside a 0.91 x 1.22 m x 0.60 m healing chamber with a plastic cover, 55% shade cloth, and a supplemental cool-mist humidifier as described in Rivard et al. (2010a). Following graft union formation, approximately 10 days after grafting, all tomato seedlings were removed from the healing chamber and grown in the greenhouse for approximately 14 days prior to field transplanting.

Experimental Design and Data Collection

A total of six experiments were conducted at four sites in 2011 and 2012. All six trials contained five identical treatments and were planted in a randomized complete block design with four replications. Five trials were located in high tunnels and treatments included: nongrafted 'BHN 589', 'BHN 589' grafted onto Maxifort using standard methods, 'BHN 589' grafted onto Maxifort rootstock with the shoot removal technique (SR, described above), 'BHN 589' grafted onto 'Trooper Lite' rootstock and, 'BHN 589' grafted onto 'Trooper Lite' rootstock with the shoot removal technique (SR). The Reno County trial had the same rootstock/grafting method treatments, but utilized an heirloom scion, 'Cherokee Purple', and was grown in the open-field.

All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage. Fruit weight and number were recorded for each grade for each plot. All fruit larger than 5 cm were harvested at the end of each growing season and included in total yield. Above-ground vegetative growth was collected from one centrally-located plant per plot at the end of the trials. Samples were dried at 70°C for at least 96 hours and weighed to determine the effect of rootstock and scion shoot removal on above-ground biomass.

Olathe Horticulture Research and Extension Center Trials

High tunnel trials were conducted in 2011 and 2012 at the K-State Olathe Horticulture Research and Extension Center (OHREC) located in Johnson County, KS (38.884347 N, 94.993426 W). The soil type at this location consists of Chase silt loam (pH= 6.3). This research trial was conducted within the central two rows of a three season, single-bay high tunnel (Haygrove, Inc.; Ledbury, United Kingdom) measuring at 7.3m x 61m. Two replications were planted within each of the two 30-m rows. 'BHN 589' was used as a nongrafted control and as scion for the grafted treatments. Each plot contained seven plants in 2011 and six plants in 2012. In 2011 plots measured at 4.88 m^2, and in 2012 plots measured at 4.18m^2. Each of the five treatments was randomly assigned to 3.8 m plots within each of the four blocks. Cultural methods were consistent with commercial organic tomato production. In-row plants spacings were at 46 cm and rows were 1.5 m apart. In 2011 plots measured at 4.88 m^2, and in 2012 plots measured at 4.18m^2. Pelletized organic poultry manure (Chickity Doo-Doo™, Lake Mills, WI) was applied at a rate of 114.5 kg N per hectare at planting and water was applied throughout the growing season by drip irrigation. Weeds were suppressed via woven fabric mulch and plastic mulch in 2011 and 2012, respectively, and plants were trained in to a vertical stake-and-weave trellis system.

The Olathe Horticultural Research and Extension Center trials were planted on 12 May in 2011 and 23 April in 2012. In 2011, harvests occurred on 13, 19, and 26 July; 2, 9, 16, 23, and 30 August; 6, 13, 20, and 27 September; and 4 and 11 October. In 2012, harvest dates were on 19 and 26 June; 3, 10, 12, 16, 24 and 30 July; 7, 14, 21 and 28 August; 4, 12, 18 and 29 September; and 5 October.

Johnson County On-farm Trials

Trials were conducted in 2011 and 2012 at a commercial farm located in Johnson County, KS (38.76473 N, 95.008022 W) at Gieringer's Orchard (http://www.gieringersorchard.com). The soil type in this location consisted of Sibleyville loam (pH=7.7). The trial was conducted in a (9.1m x 29.3m) gothic arch high tunnel annually planted with tomatoes. This trial was managed conventionally, with a fungicide application administered after transplanting and conventional insecticides applied as needed. 'BHN 589' was used as a nongrafted control and as scion for the grafted treatments. The trial occupied the inner four rows of the high tunnel, which had eight rows total. The four replications were planted within four 15-m rows with 1 replication per row. Each of the five treatments was randomly assigned to 2.4-m length plots within each of the four blocks. Every plot contained five plants with in-row spacings at 61 cm apart and row spacings at 1.1 m. Water was applied through drip irrigation beneath fabric mulch, which suppressed weeds. Tomato plants were trained into a modified stake-and-weave trellis system with 2 cm plastic plant clips (Hydro-gardens, Colorado Springs, CO) used to hold vines to the string trellis. All treatments were transplanted into the high tunnel on 25 April in 2011 and 21 March in 2012. Fruit were harvested on 13, 16, 19, and 26 July; 2, 9, 16, 23, and 30 August; 6, 13, 20, and 27 September; and 4, 11, and 18 October 2011. In 2012, harvests occurred on 11, 19, and 26 June; 3, 10, 17, 24 and 30 July; 7, 14, 21 and 28 August; as well as 4 and 7 September.

Wyandotte County On-Farm Trial

A trial was conducted in 2012 at the Gibbs Road farm location of Cultivate Kansas City, a non-for-profit urban farming advocacy organization (http://www.cultivatekc.org) in Wyandotte

County, KS (39.057955 N, 94.678209 W). The soil type in this location is composed of a mixture of both Lagoda silt loam and Marshall silt loam (pH=6.2). The trial was conducted in a 7.3m x 2.9 m homemade quonset-style high tunnel that undergoes seasonal crop rotations. 'BHN 589' was used as a nongrafted control and as scion for the grafted treatments, and this trial was managed organically.

The four replications were located in the two central, 27.4 m rows. Each plot contained five plants with in-row spacings at 45.7 cm apart and row spacings at 1.52 m. Pelletized organic poultry manure (Chickity Doo-Doo™, Lake Mills, WI) was applied at a rate of 143.1 kg N per hectare at planting and water was applied throughout the growing season by drip irrigation. Straw mulch was applied and tomato vines were trained in to a stake-and-weave system. All treatments were transplanted into the high tunnel on 28 March. Fruit harvests occurred on 15, 18, 25, and 28 June; 2, 3, 6, 9, 13, 16, 23, 27, and 30 July; and on 2, 6, 10, 17, 21, 24, and 30 August 2012.

Reno County On-farm Trial

This trial was conducted during 2012 at a small-scale organic farm located in Reno County, KS (38.094 N, 97.7413 W). Soils consisted of Pratt-Turon fine sands (pH=5.8). This trial was managed in four rows 22 m rows in the open-field. This trial was grown using organic practices but not located on certified organic land. Cherokee Purple was used as the nongrafted control and as scion in the grafted treatments. Vines were trellised using 60 cm (diameter) x 1.8 m tall tomato cages made from metal wire fencing. Every plot contained four plants with in-row spacings at 90 cm apart and row spacings at 1.8 m. Each replication was planted in a 22 m row with a total of four rows. Water was applied through drip irrigation, and straw mulch was applied for weed suppression. All treatments were transplanted into the field on 20 April. Harvesting occurred on 2,9, 12, 15, 18, 20, 23, 25, 29, and 31 July; 2, 5, 9, 12, 16, and 20 August; 7, 16, and 21 September; and finally on 12 October, 2012.

Statistical Analysis

The data from each location/year were treated similarly but were analyzed independently. All data were analyzed using analysis of variance (PlotIt, Scientific Programming Enterprises, Haslett, MI), and where significant treatment effects were identified, a mean separation test was carried out using an *F* protected least significant difference (LSD) test. Total (both marketable and culled fruit) and marketable yield were converted to reflect tonnes/hectare (t/ha) in the table.

RESULTS AND DISCUSSION

Olathe Horticulture Research and Extension Center Trials

In the Olathe Horticulture Research and Extension Center (OHREC) trials (**Tables 2.1-2.2**), standard grafting with Maxifort and Trooper Lite rootstocks significantly increased yield compared to nongrafted plants ($P<0.05$). Total yield increases ranged from 41% to 44 %, and 57% to 99% in 2011 and 2012, respectively. Fruit size and number of grafted treatments were significantly affected ($P<0.05$) compared to the control plots with the exception of total fruit number in 2012. Compared to the nongrafted control average total and marketable fruit size was increased with the implementation of both standard and SR grafting on Trooper Light. Average total and marketable fruit size was increased with both standard and SR grafting on Maxifort in 2012 but with SR grafting on Maxifort in 2012. In 2011, percent marketability based on both weight and volume was not significantly affected by rootstock and/or grafting method (**Table**

2.1). In 2012, nongrafted plants produced significantly less marketable fruit (**Table 2.2**; $P<0.05$).

Johnson County On-farm Trials

At the Johnson County on-farm location, significant increases in yield were seen in 2011 and 2012 using both standard and SR grafting on Maxifort and 'Trooper Lite' rootstocks but the benefit of grafting was much more dramatic in 2011 (**Tables 2.3-2.4**). In 2011, increases in marketable and total fruit yield for all grafted treatments were 98% to 126% higher than the nongrafted control plots ($P<0.05$). In contrast, total fruit yield increases in 2012 ranged from 18% to 25% but were still statistically significant compared to nongrafts (**Table 2.4**; $P<0.05$). Both standard and SR grafting on both rootstocks significantly increased fruit size and number in 2011 and fruit number in 2012 compared to the nongrafted controls (**Table 2.3** & 2.**4**; $P<0.05$). Fruit marketability was significantly higher in 2011 with both SR and standard grafting on Maxifort and 'Trooper Lite' rootstocks and were higher in 2012 when calculated by weight (**Table 2.4**; $P<0.05$).

Wyandotte County On-farm Trial

In the Wyandotte County on-farm trial no statistically significant differences in marketable or total yield between treatments (**Table 2.5**). However, yield increases ranged from 21% to 30% compared to the control. Maxifort had significantly higher fruit number ($P<0.05$), but average fruit size was not affected. It is not clear why statistically significant yield effects were not seen in this high tunnel study as compared to similar trials at OHREC and the Johnson County commercial farm.

Reno County On-farm Trial

In the Reno County on-farm trial the top performing treatments in regards to final total and marketable yield were grafted plants with shoots removed (SR) during the grafting procedure (**Table 2.6**). However, yield of SR-grafted plants were not significantly different than plants grafted using the standard method. Plants grafted with Maxifort that had shoots removed had the greatest marketable and total fruit size and plants grafted with 'Trooper Lite' had the lowest average fruit size, although it was not significantly different from the nongrafted control ($P<0.05$). Grafting had no statistically significant effect on marketability in this trial.

Effect of grafting method and rootstock on shoot biomass

Significant effects of grafting method and/or rootstock were seen on shoot biomass in four of the six trials ($P<0.05$; **Table 2.7**). Standard tube grafting with Maxifort significantly increased shoot growth compared to the control in all four of these trials ($P<0.05$) 'Trooper Lite' increased shoot biomass compared to the nongrafted control in only one study (2012 Johnson County on-farm trial). Similarly, plants grafted with SR-Maxifort treatments had significantly increased biomass in two trials ($P<0.05$) whereas SR-'Trooper Lite' plants had similar plant growth to nongrafted controls in all trials. This data indicates that plant vigor by plants grafted with Maxifort was higher than plants grafted with 'Trooper Lite' although statistical differences between the two rootstocks were seen in only one out of the six trials when comparing the standard-grafted treatments.

Grafting With Inter-specific Rootstocks for High Tunnel Production in the Great Plains

Grafting with inter-specific rootstock significantly increased yield in five of the six tomato trials reported here ($P<0.05$) and this effect was particularly pronounced in the high tunnel trials. Increases in yield when comparing the standard grafted plants with nongrafted controls ranged from 18% to 126%. The average yield increase when Maxifort rootstock was utilized was 53% across all the trials. Similarly, the average yield benefit with the use of 'Trooper Lite' rootstock was 51%. This data indicates that both rootstocks were successful at increasing fruit yield for tomato growers in the Great Plains and were similar when compared to each other using the standard tube-grafting technique. It is not clear why the effect of grafting was so pronounced in 2011 as compared to 2012 at the Johnson County on-farm location. Nongrafted marketable and total yields were particularly low in the Johnson County on-farm trial in 2011 as compared to 2012. This data suggests the ability of grafted plants to perform well during years with poor growing conditions for tomato production.

Effect of Shoot Removal on Grafted Plant Performance

The effect of shoot removal on plant performance was not as consistent as grafting across all six of the trials. However, some trends can be observed, particularly as fruit yield of grafted plants is related to rootstock vigor. Overall, the effect of shoot removal reduced performance of the grafted plants as it relates to final plant yield (**Tables 2.1-2.6**). Across all of the six trials, observations can be made for both total and marketable fruit yield, comprising twelve comparisons in total. Out of the twelve comparisons, Maxifort increased fruit yield in eight of these (**Tables 2.1-2.4**) and was not significant at two locations (**Tables 2.5-2.6**). Similarly, when Maxifort was grafted using the shoot removal (SR) technique, significant increases were seen in seven of the twelve comparisons for total and marketable fruit yield. When comparing 'Trooper Lite' in the same manner, significant yield increases were seen in seven of twelve comparisons for the standard grafting technique, but only five of the twelve comparisons for the SR-grafted plants ($P<0.05$). Interestingly, in four comparisons (**Tables 2.3-2.4**), SR-grafted 'Trooper Lite' had significantly lower fruit yield than standard-grafted 'Trooper Lite' plants ($P<0.05$) whereas all plants grafted with Maxifort had statistically similar fruit yield. These results suggest that 'Trooper Lite' was penalized by the shoot removal technique as this procedure reduced fruit production in four of the trials reported here. One explanation for this could be a lack of vigor by 'Trooper Lite' as compared to Maxifort. Removal of the shoot during the grafting procedure results in a smaller transplant at planting with fewer and/or smaller developed leaves. These plants are therefore required to grow faster in order to catch up to their counterparts grafted with the standard technique. Shoot biomass was significantly increased in four of the six trials by Maxifort and only one of the six trials by 'Trooper Lite' (**Table 2.7**; $P<0.05$) when the standard grafting technique was utilized. This indicates that Maxifort increased vigor that was not provided by 'Trooper Lite'. For future studies, a comparison of non- or self-grafted plants that have undergone the shoot removal process would be advantageous for determining its effect upon mature plant yield.

An important question concerning the utilization of grafted plants is to compare crop yields of grafted and nongrafted plants, particularly as they relate to early vs. mid- and late-season production. For this reason, total cumulative plant yield is presented in **Figures 2.2-2.4**. Because grafted plants with their shoots removed may be smaller at the time of planting, they may reduce the early-season yield as compared to plants with the standard grafting method. An examination of the cumulative yield curves indicate that although SR-grafted plants may perform similarly when final yield is tabulated at the end of the year, it could have negative effects on early and mid-season production.

At the Olathe Horticulture Center in 2011, Trooper Lite provided the highest early-season production, including treatments where the SR technique was performed. Conversely, SR-Maxifort plants had lower early-season yields and caught up with standard grafted plants 90 days after planting (**Fig 2.2A**). In 2012, early season yield was fairly similar across all treatments, but SR-Trooper Lite had lower cumulative yield than the other treatments until 70 days after planting when yields were comparable among all treatments (**Fig 2.2B**). Similar to 2011, the benefit of using the SR-Maxifort and SR-Trooper Lite plants in 2012 was not equal to the standard-grafted plants until 125 days after planting (**Fig 2.2B**). At the Johnson County on-farm location, both SR-grafted treatments showed a dramatic lag in yield as compared to standard-grafted plants in both years (**Fig 2.3A-B**). Similarly to the OHREC trials, cumulative fruit yield increased among the SR-grafted plants during the mid- and late-season (**Fig 2.3A**), and no statistical differences were seen between the standard- and SR-grafted plants in 2011 (**Table 2.3**; $P<0.05$). In 2012, however, cumulative fruit yield of SR-grafted plants was not able to catch up to standard-grafted plants (**Fig 2.3B**) and statistically significant differences were seen (**Table 2.4**). Interestingly, in the 2012 study, the nongrafted plants produced much higher yield early in the season and then provided little additional fruit production in the mid- and late-season (**Figure 2.3B).** In the Wyandotte County trial, a pronounced yield lag can be observed by the SR-Trooper Lite and SR-Maxifort treatments. Plants grafted with the shoot removal technique had lower yields than all other treatments until 100 days after planting and final total yield was statistically similar to nongrafted plants.

Conversely to the other trials reported here, the SR-grafted plants did not reduce early season production in the Reno County on-farm trial, and the data suggests that these plants benefited from shoot removal (**Table 2.7**; *P*<0.05). It should be noted that in contrast to the sites, this trial utilized an heirloom, indeterminate cultivar and the plants were grown in cages at a much lower planting density and in the open-field. It could be suggested that the added leader of the plant as a result of shoot removal was successful at increasing leaf area and therefore overall crop vigor and yield. Furthermore, the increased plant spacing allows for the efficient use of larger vegetative growth.

CONCLUSIONS

These trials indicate that tomato grafting is a viable and potentially profitable practice for organic/small-acreage growers in Kansas. Previous reports demonstrated that profitable yield increases may occur in grafted vegetable crops, when few biotic stressors are present (Ruiz and Romero, 1999; Yetisir and Sari, 2003). Our study suggests that grafting with inter-specific hybrid rootstocks, Maxifort and 'Trooper Lite', increases fruit yield when little disease pressure is evident in high tunnels, which are commonly utilized for tomato production on small farms (Carey et al, 2009). Both rootstocks conferred a significant increase in yield compared to the nongrafted plants when the standard tube-grafting method was utilized.

The effect of shoot removal was less consistent across the six trials and seems to be affected by rootstock cultivar. Final yield was not affected when Maxifort rootstock was grafted using the SR technique as compared to Trooper Lite. Maxifort is an especially vigorous rootstock and has shown yield increases in previous studies (Rivard and Louws 2010b), and particularly during the later part of the season (Rivard and Louws, 2008). However, both rootstocks exhibited a lag in production during the early harvest period (up to 100 days after planting). Removing the shoot was observed to reduce early season plant growth especially in the first 2 to 3 weeks after transplanting (data not shown). This suggests that the required re-growth of the scion tissue after removing the shoots resulted in lower yields than standard grafting methods; therefore, Trooper Lite rootstock grafts may require a longer recuperation period while Maxifort-grafted plants may recover more quickly. Plant growth effects were significantly higher among the standard Maxifort grafts than the nongrafted plants whereas Trooper Lite was not as vigorous which would explain the rootstock effects seen in our study. The added growth rate of the plants grafted with Maxifort was able to compensate for the required re-growth needed for the removed shoots. Rootstock vigor may be an important consideration for growers wishing to utilize the SR technique.

This data suggests that grafting could be a highly advantageous technology for high tunnel growers in the Great Plains. Grafting is a beneficial option in terms of yield for growers, but growers interested in on-farm grafting (as opposed to purchasing grafted plants) may discover many challenges in terms of grafted propagation. Therefore, simplified techniques that require less intensive management are critical for adoption of grafting for tomato growers. Although the shoot removal technique may not be a consistent method in terms of mature plant yield, it may be a valuable technique when used with certain rootstocks to boost yield and simplify the grafting procedure.

Table 2.1 Tomato fruit yield[v] and marketability of grafted and nongrafted ‘BHN 589’ grown using organic practices in a high tunnel at the Olathe Horticultural Research and Extension Center in 2011[w].

	Marketable fruit yield			Total fruit yield			% Marketability[y]	
Grafting method	(t/ha)[x]	Size (g)	No. (10^3/ha)	(t/ha)	Size (g)	No. (10^3/ha)	Weight	Number
Nongrafted	94.0 a	152 a	615.6 a	108.8 a	146 a	745.3 a	86.2	82.6
Maxifort (standard)	135.4 b	164 ab	827.3 b	147.6 b	158 ab	931.3 b	91.8	88.8
Maxifort (SR)[z]	125.7 ab	170 ab	739.1 ab	144.4 b	165 b	877.0 ab	87.1	84.3
Trooper Lite (standard)	135.6 b	174 b	778.1 ab	153.9 b	169 b	907.2 ab	87.8	85.5
Trooper Lite (SR)	120.9 ab	176 b	686.8 ab	136.9 ab	171 b	800.6 ab	88.3	85.7

[v] The experiment was set up in a randomized complete block design with four replications and seven plants per treatment per block. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage. Total and marketable yield per plot were recorded every 5-10 days. Values followed by the same letter in the same column are not significantly different according to a protected Tukey’s w-procedure ($\propto = 0.05$). No letters means effects were not statistically significant.

[w] The duration of the growing season was from12 May to 11 October 2011.

[x] Conversion factor from pounds per plot to seasonal tonnes per hectare is 0.93.

[y] Percent marketability was determined by dividing the marketable yield (weight, size, number) by the total yield (weight, size, number).

[z] Shoot removal method (SR).

Table 2.2 Tomato fruit yield[v] and marketability of grafted and nongrafted ‘BHN 589’ grown using organic practices in a high tunnel at the Olathe Horticultural Research and Extension Center in 2012[w].

	Marketable fruit yield			Total fruit yield			% Marketability[y]	
Grafting method	(t/ha)[x]	Size (g)	No. (10^3/ha)	(t/ha)	Size (g)	No. (10^3/ha)	Weight	Number
Nongrafted	81.1 a	118 a	697.9 a	116.0 a	108 a	1078.8	75.8 a	71.0 a
Maxifort (standard)	150.6 b	150 b	1002.8 b	182.0 b	143 b	1275.5	87.5 bc	84.3 b
Maxifort (SR)[z]	153.0 b	165 b	913.1 ab	190.2 b	157 b	1196.0	85.4 b	81.6 b
Trooper Lite (standard)	161.6 b	155 b	1041.7 b	189.0 b	150 b	1255.2	91.6 c	89.5 b
Trooper Lite (SR)	147.8 b	157 b	944.8 ab	181.8 b	149 b	1223.5	88.9 bc	85.2 b

[v] The experiment was set up in a randomized complete block design with four replications and six plants per treatment per block. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage. Total and marketable yield per plot were recorded every 3-7 days. Values followed by the same letter in the same column are not significantly different according to a protected Tukey’s w-procedure ($\propto = 0.05$). No letters means effects were not statistically significant.

[w] The duration of the growing season was from 23 April to 5 October 2012.

[x] Conversion factor from pounds per plot to seasonal tonnes per hectare is 1.31.

[y] Percent marketability was determined by dividing the marketable yield (weight, size, number) by the total yield (weight, size, number).

[z] Shoot removal method (SR).

Table 2.3 Tomato fruit yield[v] and marketability of grafted and nongrafted 'BHN 589' grown using conventional practices in a high tunnel at a commercial farm[z] in Johnson County, KS in 2011[w].

	Marketable fruit yield			Total fruit yield			% Marketability[y]	
Grafting method	(t/ha)[x]	Size (g)	No. (10^3/ha)	(t/ha)	Size (g)	No. (10^3/ha)	Weight	Number
Nongrafted	68.8 a	123 a	558,3 a	78.1 a	117 a	665.2 a	87.7 a	83.6 a
Maxifort (standard)	155.2 c	158 b	990.3 b	159.9 c	154 b	1041.2 bc	97.0 b	95.0 b
Maxifort (SR) [z]	134.2 bc	154 b	876.2 b	139.3 bc	151 b	925.7 abc	96.4 b	94.6 b
Trooper Lite (standard)	149.5 c	145 b	1039.1 b	154.7 c	140 b	1116.6 c	96.6 b	93.1 b
Trooper Lite (SR)	111.9 b	148 b	757.1 ab	116.4 b	144 b	810.9 ab	96.1 b	93.2 b

[v] The experiment was set up in a randomized complete block design with four replications and five plants per treatment per block. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage.
Total and marketable yield per plot were recorded every 7 days. Values followed by the same letter in the same column are not significantly different according to a protected Tukey's w-procedure ($\propto = 0.05$). No letters means effects were not statistically significant.

[w] The duration of the growing season was from 25 April to 18 October 2011.

[x] Conversion factor from pounds per plot to seasonal tonnes per hectare is 1.30.

[y] Percent marketability was determined by dividing the marketable yield (weight, size, number) by the total yield (weight, size, number).

[z] Shoot removal method (SR).

Table 2.4 Tomato fruit yield[v] and marketability of grafted and nongrafted ‘BHN 589’ grown using conventional practices in a high tunnel at a commercial farm[y] in Johnson County, KS in 2012[w].

	Marketable fruit yield			Total fruit yield			% Marketability[y]	
Grafting method	(t/ha)[x]	Size (g)	No. (10^3/ha)	(t/ha)	Size (g)	No. (10^3/ha)	Weight	Number
Nongrafted	163.6 ab	154	1025.0 ab	171.7 ab	145	1190.5 ab	95.3 b	86.0
Maxifort (standard)	200.6 c	164	1203.3 b	214.2 c	154	1394.3 b	93.4 ab	87.0
Maxifort (SR)[z]	183.2 bc	161	1112.9 b	201.7 bc	155	1301.0 ab	90.1 a	86.3
Trooper Lite (standard)	192.9 bc	170	1099.6 b	208.3 c	158	1315.3 b	92.5 ab	84.0
Trooper Lite (SR)	142.2 a	160	868.8 a	157 a	146	1083.6 a	90.5 a	81.0

[v] The experiment was set up in a randomized complete block design with four replications and five plants per treatment per block. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage.
Total and marketable yield per plot were recorded every 7 days. Values followed by the same letter in the same column are not significantly different according to a protected Tukey’s w-procedure (∝ = 0.05). No letters means effects were not statistically significant.

[w] The duration of the growing season was from 21 March to 7 September 2012.

[x] Conversion factor from pounds per plot to seasonal tonnes per hectare is 1.30.

[y] Percent marketability was determined by dividing the marketable yield (weight, size, number) by the total yield (weight, size, number).

[z] Shoot removal method (SR).

Table 2.5 Tomato fruit yield[v] and marketability of grafted and nongrafted ‘BHN 589’ grown using organic practices in a high tunnel at a commercial farm in Wyandotte County, KS in 2012[w].

	Marketable fruit yield			Total fruit yield			% Marketability[y]	
Grafting method	(t/ha)[x]	Size (g)	No. (10^3/ha)	(t/ha)	Size (g)	No. (10^3/ha)	Weight	Number
Nongrafted	103.7	211	501.6 a	118.0	179	770	87.8	65.1
Maxifort (standard)	126.3	171	742.7 b	144.0	156	929.5	87.8	79.9
Maxifort (SR)[z]	119.4	193	630.0 ab	143.5	174	820.5	83.2	76.8
Trooper Lite (standard)	125.6	182	701.1 ab	153	163	943.5	82.1	74.3
Trooper Lite (SR)	91.8	179	513.1 a	111.5	163	681.0	82.3	75.3

[v] The experiment was set up in a randomized complete block design with four replications and five plants per treatment per block. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage.
Total and marketable yield per plot were recorded every 4-7 days. Since some plants died early in the growing season, numbers were normalized to account for missing tomatoes. Values followed by the same letter in the same column are not significantly different according to a protected Tukey’s w-procedure ($\propto = 0.05$).
No letters means effects were not statistically significant.

[w] The duration of the growing season was from 28 March to 30 August 2012.

[x] Conversion factor from pounds per plot to seasonal tonnes per hectare is 1.30.

[y] Percent marketability was determined by dividing the marketable yield (weight, size, number) by the total yield (weight, size, number).

[z] Shoot removal method (SR).

Table 2.6 Tomato fruit yield[v] and marketability of grafted and nongrafted ‘Cherokee Purple’ grown using organic practices in an open field at a commercial farm in Reno County, KS in 2012[w].

	Marketable fruit yield			Total fruit yield			% Marketability[y]	
Grafting method	(t/ha)[x]	Size (g)	No. (10^3/ha)	(t/ha)	Size (g)	No. (10^3/ha)	Weight	Number
Nongrafted	30.8 a	180 ab	173.2	45.7 a	171 a	268.0	65.5	62.8
Maxifort (standard)	50.0 ab	185 ab	270.4	58.4 ab	181 ab	348.8	79.0	77.3
Maxifort (SR)[z]	57.2 b	202 b	286.4	78.3 b	197 b	366.0	79.3	78.0
Trooper Lite (standard)	41.2 ab	167 a	244.0	57.5 ab	171 a	324.2	74.0	75.0
Trooper Lite (SR)	54.8 b	185 ab	280.8	68.9 ab	192 ab	359.2	79.0	77.5

[v] The experiment was set up in a randomized complete block design with four replications and five plants per treatment per block. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards including presence of fruit diseases, blossom end rot, and/or pest damage.
Total and marketable yield per plot were recorded every 4-7 days. Since some plants died early in the growing season, numbers were normalized to account for missing tomatoes. Values followed by the same letter in the same column are not significantly different according to a protected Tukey’s w-procedure ($\propto = 0.05$). No letters means effects were not statistically significant.

[w] The duration of the growing season was from 20 April to 12 October 2012.

[x] Conversion factor from pounds per plot to seasonal tonnes per hectare is 2.71.

[y] Percent marketability was determined by dividing the marketable yield (weight, size, number) by the total yield (weight, size, number).

[z] Shoot removal method (SR).

Table 2.7 Shoot biomass[v] (g) of grafted and nongrafted plants using two grafting methods and two rootstocks from six high tunnel and open-field trials in Kansas.

	Olathe Horticulture R&E Center [w]				Johnson County [x]				Wyandotte County [y]	Reno County [z]
Grafting method	2011		2012		2011		2012		2012	2012
Nongrafted	408.8	a	334.3	a	268.5	a	346.8	a	301.3	1189.0
Maxifort (standard)	577.8	b	492.0	b	470.3	b	535.5	b	536.3	1132.5
Maxifort (SR)	525.8	ab	480.8	b	421.0	ab	588.5	b	441.7	1101.8
Trooper Lite (standard)	370.8	a	455.8	ab	305.5	ab	496.0	b	539.3	1246.0
Trooper Lite (SR)	467.0	ab	435.5	ab	404.0	ab	379.0	a	311.7	1118.5

[v] Above-ground vegetative growth was collected from one centrally-located plant per plot at the end of the trials. Samples were dried at 70°C for at least 96 hours and weighed to determine the effect of rootstock and scion shoot removal on above-ground biomass. Values followed by the same letter are not significantly different according to a protected Tukey's w-procedure ($\propto = 0.05$). No letters means effects were not statistically significant.

[w] Organic high tunnel trial with 'BHN 589' scion and for nongrafted control

[x] Conventional high tunnel trial with 'BHN 589' scion and for nongrafted control

[y] Organic high tunnel trial with 'BHN 589' scion and for nongrafted control

[z] Organic open-field trial with 'Cherokee Purple' scion and for nongrafted control.

Figure 2.1 Grafted seedlings exhibiting the two grafting techniques – shoot removal (foreground) and standard tube grafting (background) inside of a healing chamber. Seedlings were kept inside a chamber with 80-95% humidity in this treatment.

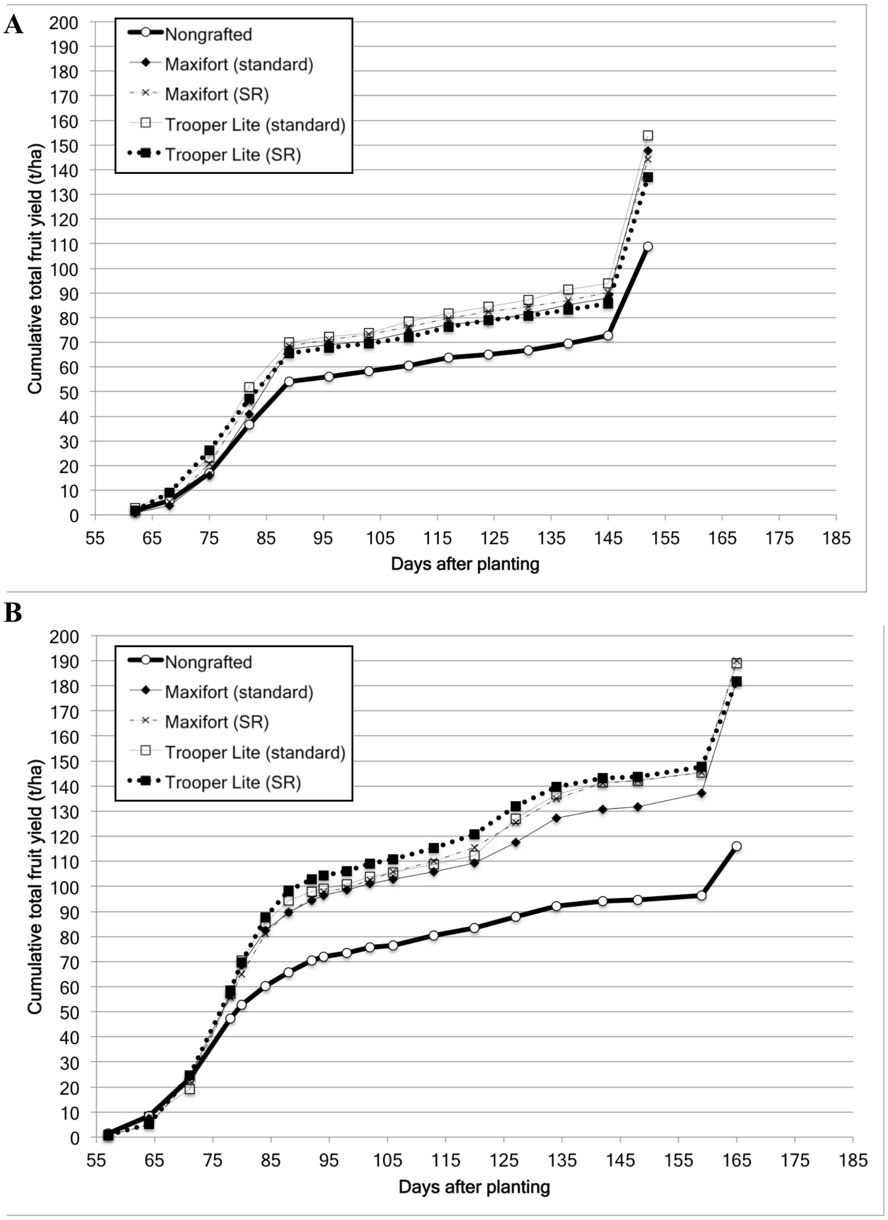

Figure 2.2 Cumulative total fruit yield of grafted and nongrafted ‘BHN 589’ grown using organic practices in a high tunnel at the Olathe Horticulture Research and Extension Center during (**A**) 2011 and (**B**) 2012 growing seasons. Grafting treatments include grafting procedure (standard and SR) and two rootstocks (cvs. Maxifort and Trooper Lite).

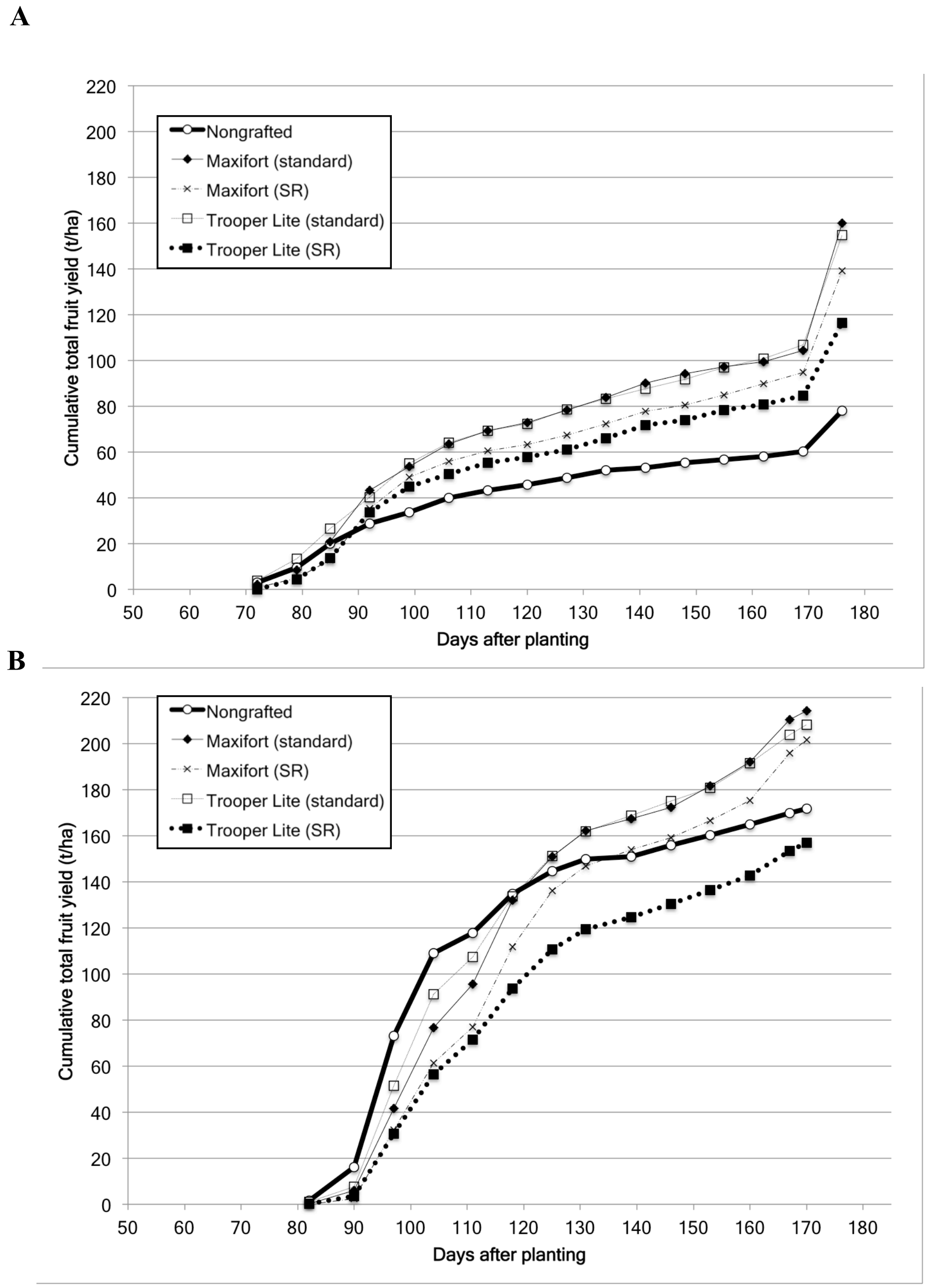

Figure 2.3 Cumulative total fruit yield of grafted and nongrafted ‘BHN 589’ grown using conventional practices in a high tunnel at a commercial farm in Johnson County, KS during (**A**) 2011 and (**B**) 2012 growing seasons. Grafting treatments included grafting procedure (standard and SR) and two rootstocks (cvs. Maxifort and Trooper Lite).

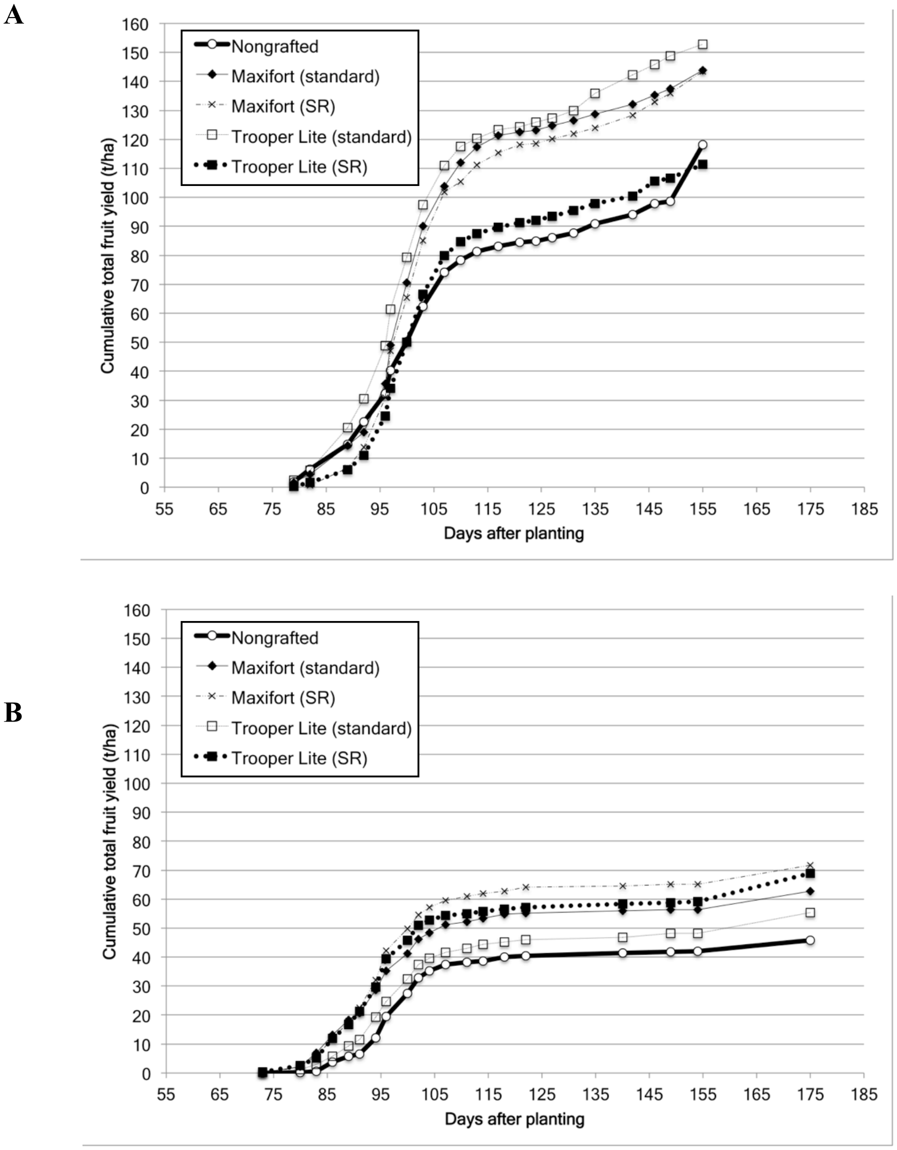

Figure 2.4 Cumulative total fruit yield of grafted and nongrafted tomatoes grown using organic practices in (**A**) a high tunnel at a commercial farm in Wyandotte County, KS and (**B**) an open field at a commercial farm in Reno County, KS during 2012 growing season. Grafting treatments standard tube-grafting technique and when shoot/meristem was removed from scion during the grafting procedure and two rootstocks (cvs. Maxifort and Trooper Lite). Scion variety was ‘BHN 589’ and ‘Cherokee Purple’ at Wyandotte and Reno County locations, respectively.

Chapter 3 - Advances in Grafting Technique and Healing Chamber Design for Tomato

INTRODUCTION

Herbaceous grafting is often applied to high-value vegetable crops such as watermelon (*Citrullus lanatus*), eggplant (*Solanum melongena*), and tomato (*Solanum lycopersicum*) due to added vigor, stress tolerance, and disease resistance (Lee, 1994 and 2003; Louws et al., 2010, 2008; Rivero et al., 2003b). Although grafting may be very useful for tomato growers in the Midwest, there is limited availability of grafted plants propagated in the US for commercial tomato fruit production. As a result, more than 30 million vegetable plants are currently imported into the United States from specialty nurseries in Canada (Kubota et al., 2008). Although a current and future market for grafted tomato plants exists, very few propagators in the United States have started grafting at a large scale. The limited supply has increased interest by both large and small tomato producers in grafting their own plants as well as grafting plants to sell. The most popular grafting technique for tomato is the tube grafting technique (also known as splice grafting or Japanese top-grafting) due to its efficiency and simplicity (Oda, 1995). This process requires that the rootstock and scion (with 1.5mm – 2mm stem diameters) are cut at approximately 60° to 75° angles, held together with a silicon grafting clip, then placed in an environment ("healing chamber") with high humidity and low light in order to promote a connection between the vascular tissues and prevent scion wilt (Oda, 2007; Davis et al., 2008).

Healing chamber management can be difficult and has been problematic for tomato growers that are experimenting with grafting, as seen in studies in North Carolina and Pennsylvania (Groff, 2009; O'Connell et al., 2009). Not only do healing chambers require increased labor and management, but also add to the overall cost of producing a grafted transplant as it requires additional materials and labor – accounting for 6.1% to 6.5% of the total added cost of grafting (Rivard et al., 2010c). Based on Asian grafting nurseries, the current recommended temperature range for healing chambers is 28°C and 29°C, and the recommended range for relative humidity is 85 and 100% (De Ruiter Seeds, 2006; Rivard and Louws, 2006). However, systematic research has not been done to actually quantify the effects of temperature and humidity on graft survival. . Furthermore, the successful adoption of grafted propagation by small-scale tomato growers requires simple effective techniques that work within limited propagation facilities. Tomatoes may be more tolerant of higher temperature and variable relative humidity (and thereby less maintenance) than other horticultural crops such as watermelon (Johnson and Miles, 2011).

In addition to modifications to the healing chamber, removal of leaf and/or shoot tissue may prevent excessive evapotranspiration and therefore reduce or eliminate the need for microclimate management. Typically applied to woody and ornamental plants, pruning of tomato plants of leaf material may be utilized to alter fruit production (Decoteau, 1990; Bennett et al., 2012); however, pruning may be applied to aid in grafting as well. Total shoot removal (SR) would greatly reduce scion transpiration lost, while moderate leaf removal (LR) could reduce transpiration loss. Reducing leaf area via elimination of scion shoots may reduce or eliminate reliance on the healing chamber. Therefore, the overall goals of this study were as follows: (i) to determine how healing chamber design (supplemental humidity and covering) affects graft survival; (ii) to determine how healing chamber design affects the healing chamber environment; (iii) and to determine how scion shoot removal affects graft survival in different healing chambers.

MATERIALS AND METHODS

Experiments were conducted at two greenhouse locations: Throckmorton Plant Sciences Center at Kansas State University (Manhattan, KS; http://www.hfrr.ksu.edu/p.aspx?tabid=38) and the K-State Olathe Horticulture Research and Extension Center (OHREC) located in Johnson County, KS (38.884347 N, 94.993426 W). All experiments were conducted in a split-plot randomized complete block design (RCBD) layout with three and four replications over time at the Manhattan and Olathe locations, respectively. The main plot factor was chamber design (described below), with four chamber designs tested in Manhattan and five tested in Olathe. The sub plot factor was grafting method, with standard and shoot removal (SR) techniques tested in Manhattan and standard, SR, and leaf removal (LR) tested in Olathe. Those methods are described in detail below.

Grafting Methods

Plants were self-grafted by grafting back onto the original root system with a commercial scion/nongrafted cultivar, 'Cherokee Purple' (Johnny's Selected Seeds; Winslow, ME USA). Self-grafting allows that the plants experience the grafting process without adding variables such as genetic incompatibility and/or inconsistent rootstock and scion angles during the grafting procedure. Using this method, we were able to focus upon the influence of environmental factors post-grafting.

During grafting, standard, shoot removal (SR), or leaf removal (LR) techniques were applied to scion shoots. The standard grafted plants retained all meristematic and foliar tissue, while the SR grafted plants were cut 1-2cm above the scion cotyledon. The LR technique is a middle-ground approach, where most mature leaf tissue (70-80%) was severed but the plant meristem remained intact.

Plants were grafted on-site at each location with trained personnel and careful steps were taken to reduce bias between individuals. Each person performing the grafting process grafted the same proportion of plants for each chamber and grafted an equal number of plants within each sub-plot treatment (grafting method).

Healing Chamber Design

Five chamber designs, 'humidifier', "plastic", "shade", "perforated plastic" and a no-chamber control ("none") were tested in Olathe. All treatments except perforated plastic were tested in Manhattan. The 'humidifier' chamber was built to specifications as described in Rivard and Louws, 2010 and is typical for small-scale propagators grafting more than 5000 plants per batch. It included a 4 mm plastic covering that encompassed the entire chamber as well as 55% shade cloth across the top and a cool-mist humidifier (SU-2000, Sunpentown, City of Industry, CA) located outside of the chamber. The humidifier delivered water vapor via PVC tubing (3 cm diameter). The 'plastic' chamber was identical to the 'humidifier' chamber except that a humidifier was not utilized. In both the "humidifier" and "plastic" treatments, 2 cm of water was added and maintained in the chamber floor for additional humidity. The 'shadecloth' chamber was covered with 55% shade cloth and lacked standing water or a humidifier. The 'none' chamber was completely vulnerable to greenhouse conditions with no environmental controls. Trays of grafted plants were placed on top of upside down clean propagation (web) trays to elevate grafted plants 5 cm above the floor of the chamber to keep them out of water where plastic coverings were utilized. Upside-down trays were arranged in the same manner to all treatments within the experiment in order to reduce bias that may be caused by tray elevation within the greenhouse. All healing chambers were built to dimensions of 0.91 x 1.22 m x 0.60 m using plastic lumber with steel wire hoops for holding plastic off of the plants. All chambers included a standardized frame with 2.5 cm x 13.6 cm plastic lumber whereby the width of the board (13.6 cm) was used to create a sidewall for the chamber. Holes were drilled into the top of the board to accommodate insertion of the steel wire on that was placed vertically into the top edge. Nine gauge wire was cut to equal lengths and inserted so as to permit a small chamber 60 cm tall at the peak. **Figure 3.1** displays the layout and appearance of the exterior of these treatments at the Manhattan location during one replication.

Healing Chamber Management

For each replication and across both studies, the grafting day was indicated as Day 0. On Day 0 for each replication, 150 plants from each grafting method were placed in each healing chamber. In Manhattan, 150 standard grafted and 150 SR grafted plants were placed in each of the four chamber designs, for a total of 1200 plants. In Olathe, 100 standard grafted, 100 SR, and 100 LR were placed in each of the five chambers. The ‘humidifier’ and ‘plastic’ treatments employed full shadecloth coverings and were briefly vented daily until Day 5, when the shade cloth was turned back halfway to provide partial light exposure.. The humidifier was removed from the ‘humidifier’ treatment on Day 7. All plants were removed from the chambers on Day 8 and watered daily.

Environmental Data Collection and Analysis

Environmental conditions within each chamber were monitored via temperature and relative humidity data loggers (EL-USB-2-LCD, Lascar Electronics, Erie, PA). A logger was placed among the seedlings in the center of each chamber and recorded environmental data at thirty minute intervals. The data loggers were activated once all seedlings were placed within the chambers and synced by using a delayed start function. Temperature and relative humidities from Day 0-8 averages, minimums, and maximums were analyzed using analysis of variance (PlotIt, Scientific Programming Enterprises, Haslett, MI). Where significant treatment effects were identified, a mean separation test was carried out using an *F* protected least significant difference test. In order to observe daily fluctuation in temperature and relative humidity for each of the healing chamber treatments, the average across replications for relative humidities and temperatures during the first full day after grafting for each replication (Day 1) were calculated (**Figures 3.2-3.3)**.

KSU Greenhouse, Manhattan, KS

Replication 1 was seeded on 22 January 2013 and subsequently transplanted into 50-cell trays on February 8. Grafting of the first replication took place on 23 February. Replication 2 was seeded on 31 January, transplanted on 15 February, and grafted on 1 March. Replication 3 was seeded on 23 February, transplanted on 13 March, and grafted on 28 March.

OHREC Greenhouse, Olathe, KS

Replication 1 was seeded on 1 February 2013 and transplanted on 15 February. Grafting of Replication occurred on 1 March. Replication 2 was seeded on 22 February, transplanted on 1 March, and grafted on 15 March. Replication 3 was seeded on 1 March, transplanted on 15 March, and grafted on 25 March. Replication 4 was seeded on 15 March, transplanted on 29 March, and grafted on 8 April.

Survival Ratings

Throughout the two-week period of each replication, wilt ratings were recorded for each treatment, although this data is not shown. On day 14, plant survival was observed and recorded. All survival data were analyzed in SPSS (IBM, Armonk, NY) and showed no significant deviation from variance homogeneity; additionally, skewness and kurtosis statistics showed that survival data is approximately normal. Percent survival were calculated and analyzed using analysis of variance (PlotIt, Scientific Programming Enterprises, Haslett, MI). Where significant treatment effects were identified, a mean separation test was carried out using an *F* protected least significant difference test.

RESULTS AND DISCUSSION

Effect of chamber design on relative humidity and temperature

Relative humidity was highly impacted by healing chamber design in both studies. At the Manhattan location, the 'plastic' and 'humidifier' chambers showed a significant increase ($P<0.01$) in minimum and average relative humidity compared to the 'none' and 'shadecloth' treatments (**Table 3.1**). There were no significant differences in maximum relative humidity in Manhattan. High relative humidity is common in greenhouses of this type on cloudy days and one or two particularly humid days make it difficult to assess the impact of maximum RH on grafting success. At OHREC, comparable results were observed, where the 'plastic' and 'humidifier' treatments had significantly greater average, minimum, and maximum relative humidity than the other three treatments ($P<0.01$).

Daily fluctuations in both temperature (°C) and RH (%) were observed in all chambers at both greenhouses, as shown in **Figures 3.2** and **3.3**, which represents the mean value of all replications for each site over a 12-hour period on day 1, post-grafting. As temperatures increase throughout the day, warm air expands and permits a greater water-holding capacity. If relative water vapor content in the area remains constant, the relative humidity will decrease as temperature increases. The chambers with little to no modification in humidity (‘none,’ ‘shadecloth,’ and ‘perf’) showed wider ranges in overall RH compared to ‘humidifier’ and ‘plastic’ treatments. Interestingly, higher humidity in the ‘humidifier’ treatments seemed to mediate higher temperatures, especially when related to ‘plastic’ treatments (**Figure 2B, 3B**), although this trend was not statistically significant.

Healing chamber design had few significant effects on temperature at both locations (**Table 3.2**). In Manhattan, no significant difference was observed in average, minimum, and maximum temperature. Growing conditions for both studies were ideal for grafting as late winter and early spring weather in the Midwest provides ample cloud cover to prevent healing chambers from over-heating in the greenhouse. This may have been a factor that led to high grafting success and little separation of the treatments overall. Unfortunately, incoming light measurements were not taken. Future studies of this type would benefit from this data as it could be correlated with healing chamber temperature fluctuations and overall grafting success. At the OHREC greenhouse, the ‘none,’ ‘plastic,’ and ‘humidifier’ chambers all had significantly greater average temperatures than the ‘shadecloth’ and ‘perforated plastic’ treatments but they were still all within 1 degree Celsius ($P<0.05$).

Percent Survival

In both studies, no significant interactions were observed between the treatment main effects and grafting technique sub-effects. In **Table 3.3**, plant survival ranged from 91% to 95% and no significant differences were observed between healing chamber treatments or grafting technique (**Table 3.3**). Plants in the 'none' treatment and 'shadecloth' treatment exhibited more average wilting than those in the 'plastic' and 'humidifier' chambers ($P<0.05$; **Table 3.3**), which were statistically similar to each other. These results indicate that plant stress was reduced with increasing levels of humidity.

Similar to the experiment in Manhattan, healing chamber treatments had no effect on graft survival in the Olathe study (**Table 3.4**), which ranged from 77% to 87% across the different chamber types. However, the main effects of grafting method showed that plants grafted with the leaf removal (LR) technique had higher survival percentage than the other grafting methods at 84% ($P<0.05$; **Table 3.4**) across all the chamber types.

CONCLUSIONS

Grafting tomatoes with inter-specific rootstock is an effective method for reducing the incidence and/or severity of soilborne diseases in the United States (Louws et al., 2010), but the lack of grafted plants available for purchase by small growers can be a major barrier for adoption of this technology (Kubota et al., 2008). Many small-acreage growers are very interested in performing their own grafting, but management of the healing chamber can be difficult (Groff, 2009; O'Connell et al., 2009), particularly when limited propagation facilities are available. In this study, we tested the effect of healing chamber design on environmental conditions as well as grafting success. In our study, we saw no significant effects of healing chamber design on grafting success, and plants grafted with no chamber had success rates of 81% to 91%. Similarly, we saw no effect of using a cool-mist humidifier, which is often recommended for small growers propagating their own plants (Rivard and Louws, 2010). Our data suggests that a humidifier may not be necessary and similar results were seen by Johnson and Miles (2011). The ‘shade cloth’ treatment performed very well in our studies and was also successful for tomatoes in the study reported by Johnson and Miles (2011). Growers may experiment with chamber modifications in order to reduce the risk of “over-heating” in the greenhouse and our study provides information related to the effects of chamber coverings on environmental conditions.

Another approach for lowering water stress in the scion is the removal of leaves in order to reduce transpiration within the scion tissue post-grafting. In our study, removal of scion leaves increased plant survival compared to standard controls, but removal of the shoot did not affect percent plant survival. It must be noted that self-grafting of these plants presented a best-case scenario, and No statistical interactions were seen between healing chamber design and grafting method. Removal of the shoot and meristem requires that the plant re-initiate a meristem at the cotyledon leaves in addition to healing the graft union. Furthermore, we saw in preliminary studies that SR plants are susceptible to plant death by being shaded out by standard-grafted plants (data not shown). This indicates that the plants may not possess enough stored sugars in order to heal the graft union and develop a meristem before photosynthesis is re-initiated post-grafting. The leaf removal (LR) method tested in our studies showed significant promise and plants grafted using this technique had significantly higher success rates as compared to standard- and SR-grafted plants ($P<0.05$). Leaf removal may be recommended as a way to reduce water stress in the plant, and could potentially be a way to simplify the grafting process for small-scale propagators. A clear question for future research in this area is to determine how leaf and/or shoot removal affects the performance of mature plants in the field. The re-growth required as compared to plants grafted using the standard method may delay early growth and subsequent fruit production.

Figure 3.1 Layout of healing chamber treatments for the first replication in the Manhattan, KS greenhouse. From left to right: a ‘shadecloth only’ treatment, a ‘no chamber’ treatment, a ‘plastic’ treatment, and a ‘humidified’ treatment. Subplots (grafting method) were not randomized within treatments.

Table 3.1 Effects[t] of chamber design on average relative humidity at Manhattan and Olathe greenhouse studies.

	Percent relative humidity[u]					
Effect	**Average**		**Min**		**Max**	
Manhattan, KS						
None[v]	48.4	a	17.2	a	89.8	
Shadecloth[w]	50.5	a	19.7	a	94.3	
Plastic[x]	78.9	b	35.3	b	99.3	
Humidifier[x,y]	85.1	b	37.2	b	98.5	
OHREC						
None	66.8	a	25.3	a	91.8	a
Shadecloth	69.2	a	26.9	a	93.3	a
Perf. Plastic[z]	69.2	a	25.5	a	93.3	a
Plastic	85.3	b	33.0	b	97.6	b
Humidifier	91.3	c	37.5	c	99.8	c

[t] Values followed by the same letter are not significantly different according to a protected least significant difference test (α = 0.05).

[u] Environmental conditions within each chamber were monitored via temperature and relative humidity data loggers (EL-USB-2-LCD). A logger was placed among the seedlings in the center of each chamber and recorded environmental data at 30-min. intervals. The data loggers were activated once all seedlings were placed within the chambers and synced by using a delayed start function. Relative humidity from Day 0-8 per treatment was averaged in Excel.

[v] 'None' chamber is completely vulnerable to greenhouse conditions with no environmental controls other than the upside-down web trays to elevate the trays off of the bench.

[w] 'Shadecloth' chamber was covered with 55% shade cloth with no humidity modifications.

[x] 'Humidifier' and 'plastic' treatments employed 4 mm plastic covering that encompassed the entire chamber as well as 55% shade cloth across the top; both were vented daily until Day 5, where the cloth was turned back halfway and partial shade was applied. These treatments also had 2 cm of water in the bottom of the chamber.

[y] 'Humidifier' chamber utilized a cool-mist humidifier which was removed from the treatment on Day 7.

[z] 'Perf. Plastic' was similar in conceptual design to the 'shadecloth' treatment and was only covered in white perforated plastic and with no supplemental humidity.

Table 3.2 Effects[t] of chamber design on temperature at Manhattan and Olathe greenhouse trials.

	Temperature (°C)[u]			
Effect	**Average**		**Min**	**Max**
Manhattan, KS				
None[v]	20.8		14.2	28.8
Shadecloth[w]	20.4		13.5	27.3
Plastic[x]	20.5		14.2	29.5
Humidifier[x,y]	20.4		14.2	29.7
OHREC				
None	20.8	b	10.5	35.3
Shadecloth	19.8	a	10.9	31.6
Perf. Plastic[z]	20.0	a	10.8	32.6
Plastic	20.5	b	10.4	35.8
Humidifier	20.5	b	10.4	35.1

[t] Values followed by the same letter are not significantly different according to a protected least significant difference test (α = 0.05).

[u] Environmental conditions within each chamber were monitored via temperature and relative humidity data loggers (EL-USB-2-LCD). A logger was placed among the seedlings in the center of each chamber and recorded environmental data at 30-min. intervals. The data loggers were activated once all seedlings were placed within the chambers and synced by using a delayed start function. Temperature from Day 0-8 per treatment was averaged in Excel.

[v] ‘None’ chamber is completely vulnerable to greenhouse conditions with no environmental controls other than the upside-down web trays to elevate the trays off of the bench.

[w] ‘Shadecloth’ chamber was covered with 55% shade cloth with no humidity modifications.

[x] ‘Humidifier’ and ‘plastic’ treatments employed 4 mm plastic covering that encompassed the entire chamber as well as 55% shade cloth across the top; both were vented daily until Day 5, where the cloth was turned back halfway and partial shade was applied.

[y] ‘Humidifier’ chamber utilized a cool-mist humidifier which was removed from the treatment on Day 7.

[z] ‘Perf. Plastic’ was similar in conceptual design to the ‘shadecloth’ treatment and was only covered in white perforated plastic and maintained no humidity amendments.

Table 3.3 Main effects[s] of chamber design and grafting technique upon percent survival in greenhouse study in Manhattan, KS.

Effect	**% Survival**[t]
Chamber Design	
None[u]	91
Shadecloth[v]	94
Plastic[w]	95
Humidifier[w,x]	94
$LSD_{(0.05)}$	4.7
Grafting Method	
Standard[y]	94
Shoot removal[z]	93
$LSD_{(0.05)}$	4.2

[s] Values followed by the same letter are not significantly different according to a protected least significant difference test (α = 0.05).

[t] Healthy plants were counted and divided by the total number of plants in each treatment or subplot.

[u] 'None' chamber is completely vulnerable to greenhouse conditions with no environmental controls other than the upside-down web trays to elevate the trays off of the bench.

[v] 'Shadecloth' chamber was covered with 55% shade cloth with no humidity modifications.

[w] 'Humidifier' and 'plastic' treatments employed 4 mm plastic covering that encompassed the entire chamber as well as 55% shade cloth across the top; both were vented daily until Day 5, where the cloth was turned back halfway and partial shade was applied.

[x] 'Humidifier' chamber utilized a cool-mist humidifier which was removed from the treatment on Day 7.

[y] 100% defoliation.

[z] Only mature leaves (approximately 50-60% of total leaf area) were removed upon grafting.

Table 3.4 Main effects[q] of chamber design and grafting technique upon percent survival in greenhouse study in Olathe, KS.

Effect	**% Survival**[r]	
Chamber Design		
None[s]	81	a
Shadecloth[t]	78	a
Perf. Plastic[u]	77	a
Plastic[v]	78	a
Humidifier[v,w]	87	a
$LSD_{(0.05)}$	0.077	
Grafting Method		
Standard[x]	78	a
Shoot removal[y]	79	a
Leaf removal[z]	84	b
$LSD_{(0.05)}$	0.051	

[q] Values followed by the same letter are not significantly different according to a protected least significant difference test (α = 0.05).

[r] Healthy plants were counted and divided by the total number of plants in each treatment or subplot.

[s] 'None' chamber is completely vulnerable to greenhouse conditions with no environmental controls other than the upside-down web trays to elevate the trays off of the bench.

[t] 'Shadecloth' chamber was covered with 55% shade cloth with no humidity modifications.

[u] Perf. Plastic' was similar in conceptual design to the 'shadecloth' treatment and was only covered in white perforated plastic and maintained no humidity amendments.

[v] 'Humidifier' and 'plastic' treatments employed 4 mm plastic covering that encompassed the entire chamber as well as 55% shade cloth across the top; both were vented daily until Day 5, where the cloth was turned back halfway and partial shade was applied.

[w] 'Humidifier' chamber utilized a cool-mist humidifier which was removed from the treatment on Day 7.

[x] No leaves were removed during grafting procedure.

[y] 100% defoliation.

[z] Only mature leaves (approximately 50-60% of total leaf area) were removed upon grafting.

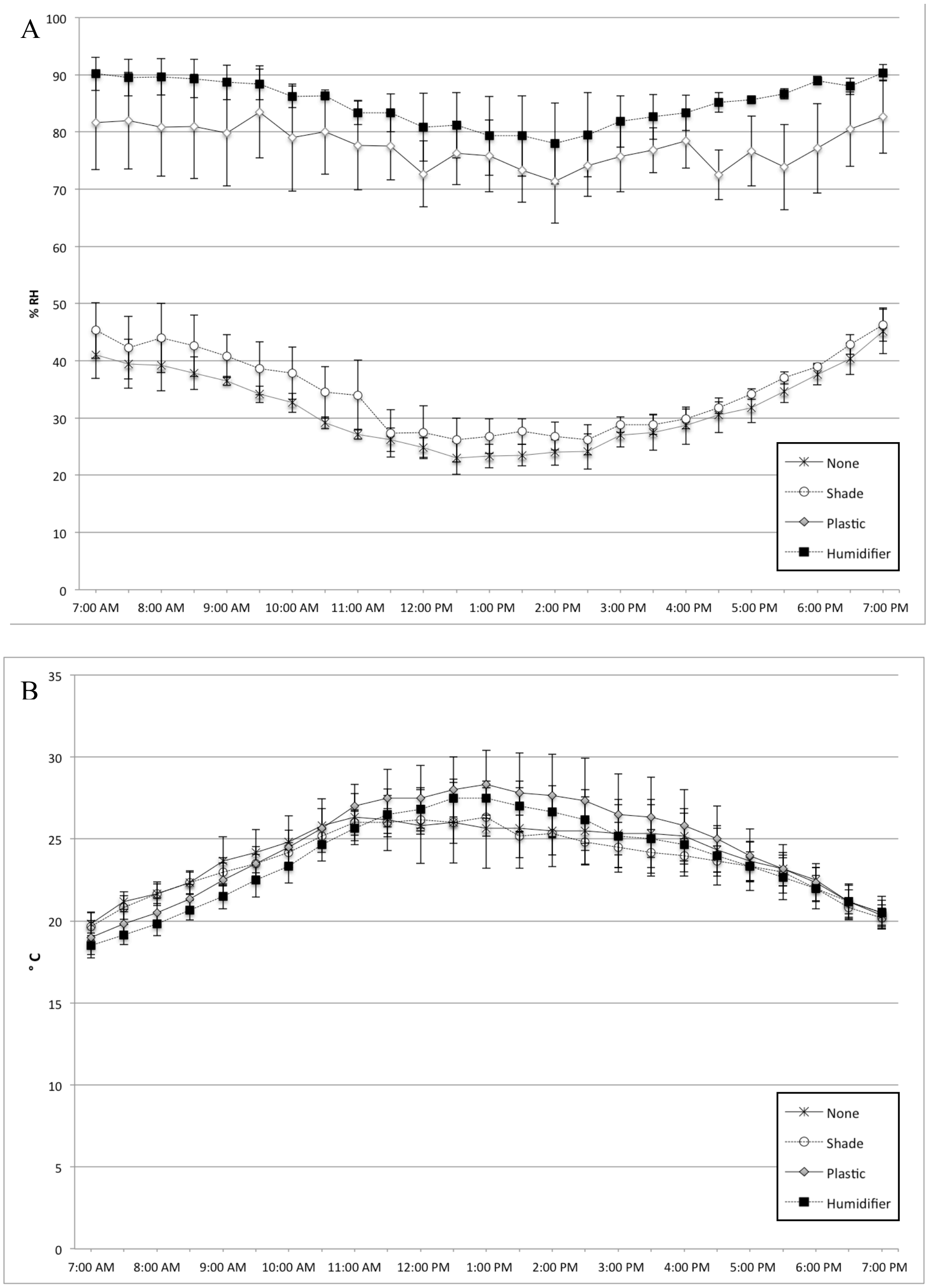

Figure 3.2 Average recordings of relative humidities (**A**) and temperatures (**B**) within different chamber treatments at a greenhouse in Manhattan, KS were obtained via data loggers during a 12-hour period following the initial grafting day. Vertical bars represent one standard error of the mean.

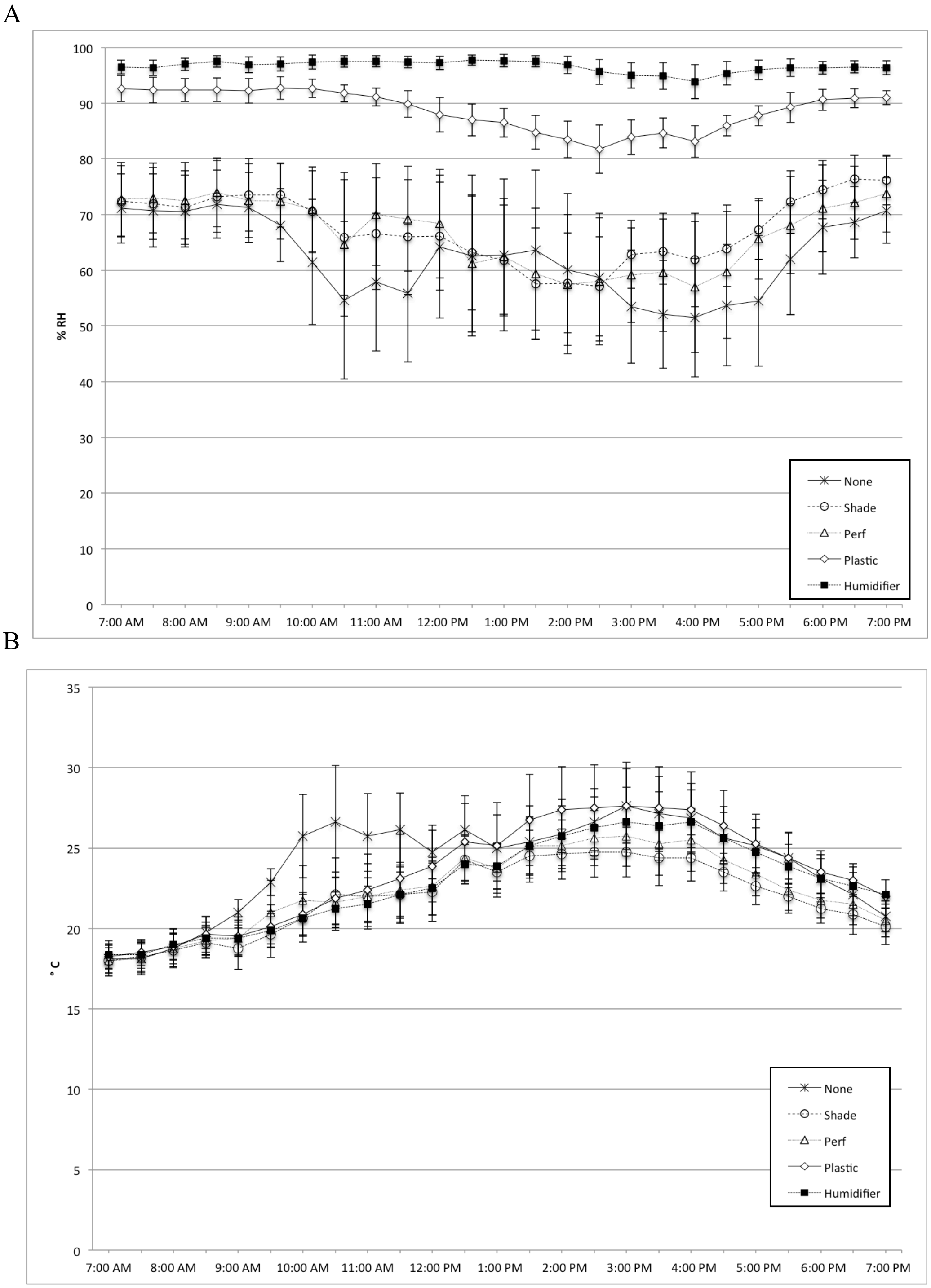

Figure 3.3Average recordings of relative humidities (**A**) and temperatures (**B**) within different chamber treatments at a greenhouse in Olathe, KS during a 12-hour period (7am-7pm) following the initial grafting day. Vertical bars represent SE.

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